# Differential responses of antioxidants and dehydrin expression in two switchgrass (*Panicum virgatum*) cultivars contrasting in drought tolerance

**Running title**: Switchgrass drought Tolerance and Antioxidant

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## Abstract

Drought stress is a major limiting factor for plant growth and development in many regions of the world. This study was designed to investigate antioxidant metabolism and dehydrin expression responses to drought stress in two switchgrass cultivars (drought tolerant Alamo, and drought sensitive Dacotah) contrasting in drought tolerance. The plants were subjected to well-watered [100% evapotranspiration (ET)] or drought stress (30%-50% ET) conditions for up to 24 d in growth chambers. Drought stress decreased leaf relative water content (RWC), increased leaf electrolyte leakage (EL), leaf malondialdehyde (MDA) content in two cultivars, but Alamo exhibited higher leaf RWC level, lower leaf EL and MDA when compared to Dacotah at 24 d of drought treatment. Drought stress also increased superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities in two cultivars, Alamo had relatively higher SOD, CAT and APX activities and greater abundance of SOD and APX isozymes than Dacotah at 24 d of drought treatment. Alamo had higher abundance of 55 KDa and 18 KDa dehydrin accumulation than Dacotah under drought treatment. Relative genes expression level of *PvCAT1, PvAPX2, PvERD and PvPIP1;5* in Alamo were significantly higher than Dacotah at 24 d of drought treatment. These results suggest that increase in antioxidant enzymes and accumulation of dehydrin were highly related with switchgrass drought tolerance. Antioxidant enzyme activity, isozyme expression and dehydrin abundance could provide a useful screening tool to identify relative drought tolerance in switchgrass cultivars.

**Keywords:** *Panicum virgatum*, drought tolerance, antioxidant, dehydrin, gene expression

## Introduction

Drought occurs in all climates and many parts of the world every year when sufficient water needed to sustain an area is not available, causing significantly impacts on plants growth, development and crop yield [1]. Worldwide losses in crop yields from drought probably exceed the losses from all other abiotic stress combined [2, 3]. Drought stresses cause oxidative stress by contributing to reactive oxygen species (ROS) such as superoxide radical ($O^{\bullet-}_2$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH\bullet$) and singlet oxygen ($^1O_2$) [4]. ROS can seriously disrupt normal metabolism through oxidative damage to nucleic acids, lipids, protein and damage membrane function [5]. However, ROS may also serve as a transduction signal during drought stress and improve stress defense mechanisms of plant [6]. ROS levels that are too low or too high affect plant growth and development, maintaining ROS levels within a moderate range is important for plants [7, 8].

Plants have developed an antioxidant defense system in response to the high level of ROS [9]. There are generally two repairing mechanisms that plants have developed to scavenge free ROS: (i) production of antioxidants or antioxidant enzymes that directly react with and scavenge ROS, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and a tocopherol; (ii) production of enzymes that regenerate oxidized antioxidants such as glutathione, glutathione reductase, ascorbate, and ascorbate reductase[10]. Previous studies provided correlative evidence that the enhanced drought tolerance of plant was associated with

72 changes in antioxidant enzymes (SOD, POD, CAT, POX and GR) and maintenance of low $H_2O_2$
73 levels [11-13]. It is known that organelles with a highly oxidizing metabolic activity or an intense
74 rate of electron flow, such as chloroplasts, mitochondria, and microbodies, are major sources of
75 ROS, different isoenzymes such as Cu/Zn-SOD, Fe-SOD, Mn-SOD, cytosol APX (cAPX), and
76 microbody APX (mAPX) have been found in different organelles [14]. The antioxidant isozymes
77 could be used as a biochemical marker to study the tolerance of plant to stress. Sen and
78 Alikamanoglu [15] found that two new POX isozyme bands were detected in all drought-tolerant
79 sugar beet mutants compared to the control, and the intensity of Fe-SOD, Cu/Zn-SOD, CAT and
80 APX isozymes were detected at different intensities among the drought-tolerant sugar beet mutants.
81 Recent study found that a new CuZn SOD iszyme OsCSD3 which encoded by LOC_Os03g11960
82 of rice, was up-regulated in response to drought, oxidative stress and salt [16].
83 Dehydrin (DHN) is a multi-family of proteins present in plants that are produced in response
84 to cold and drought stress. DHNs are hydrophilic and reliably thermostable. They are stress
85 proteins with a high number of charged amino acids that belong to the Group II Late
86 Embryogenesis Abundant (LEA) family [17, 18]. DHNs have been divided into five subclasses
87 based on their conserved amino acid sequences: the Y, S and K segments and include YnSKn,
88 SKn, Kn, YnKn and KnS sub-types[19]. It has been reported that the expression of DHN is
89 positively correlated with the tolerance to cold, drought, and salt stress [20]. DHNs play an
90 important protective role during cellular dehydration. The accumulation of DHNs was observed
91 in roots, leaves, coleoptiles, crowns and seeds under drought stress [21].
92 Switchgrass (*Panicum virgatum*) was selected as a model bioenergy crop in the United Sates
93 [22]. To avoid competition of arable lands with food crops, switchgrass will be mainly grown on
94 marginal lands, where millions of hectares of these lands are drought-affected [23]. Two distinct

95 switchgrass ecotypes are generally defined based on morphological characteristics and habitat:
96 lowland and upland. Lowland ecotypes are mostly tetraploid (2n = 4× = 36) while upland ecotypes
97 are mainly octaploid (2n = 8× = 72) or hexaploid (2n = 6× = 54) [24]. Lowland ecotypes are taller
98 and higher biomass yield, whereas upland ecotypes are shorter and less biomass yield [25].
99 Improving switchgrass yield under drought stress is one of the most important goals of plant
100 breeding. Our previous study have found that switchgrass exhibits a wide range of genetic
101 variability in drought tolerance, Alamo ranked # 4 in drought tolerance and Dacotah ranked # 48
102 among 49 switchgrass lines [26]. However, limited information is available on the gene
103 expressions in conjunction with antioxidant and dehydrin responses and isozyme alterations under
104 drought tolerance in switchgrass. Identifying and understanding the function of antioxidant
105 defense mechanisms are important for developing drought tolerant switchgrass plants. The
106 objectives of this study were to determine whether drought resistance of switchgrass cultivars
107 could be associated with antioxidant metabolism and dehydrin expression levels.
108

109 **Materials and methods**

110 **Plant materials and culture**

111 Two cultivars Alamo and Dacotah were examined in this study. Each switchgrass line was
112 propagated by splitting tillers on Mar, 10, 2014. Five tillers from each line were transplanted into
113 plastic pots (17 cm diam., 20 cm high, with four holes at the bottom for drainage) filled with 3.5
114 kg of a soil and sand mixture (soil:sand = 2:1 v/v, sand: 0.1-1.0 mm diam.). The plants were grown
115 in greenhouse with temperatures of 30±1 $^{o}$C/25±1 $^{o}$C (day/night), a 14-h photoperiod, 75% relative
116 humidity, and with photosynthetically active radiation (PAR) of approximately 500 μmol $m^{-2}s^{-1}$
117 (natural daylight supplemented with fluorescent lamps). The plants were irrigated daily, and

118 fertilizer containing N (Bulldog brand, 28-8-18, 1% ammonia N, 4.8% nitrate N, and 22.2% urea
119 N; SQM North America, Atlanta, GA) and micronutrients was applied at 0.1 lb. 1000 $ft^{-2}$ every
120 week.

121 After the plants were grown for two months and had reached the E5 developmental stage [27],
122 then moved into a growth chamber for the experiment. The chamber was set at 30/25 $^{o}$C (day/night
123 temperature), 75% of relative humidity, 14 h photoperiod, and PAR at 500 μmol $m^{-2}s^{-1}$. Plants
124 were fertilized once a week, and watered every two days until water drainage occurred at the
125 bottom of the pot at each irrigation.

126

127 **Drought stress treatment**

128 In order to determine the soil water content (SWC) of each pot more quickly, an equation of
129 linear regression between the SWC and volumetric soil moisture content (VWC) was made before
130 the drought treatment. Soil of eight pots was oven dried at 105°C for 48 h to obtain their dry
131 weights (DW). Then we added enough water to each pots, after 1 h when no water leaked from the
132 bottom of the pots, fresh weight of each pot and VWC were measured with a soil moisture meter
133 (model HH2, Delta-T Devices, Cambridge, England) and every day thereafter. SWC was
134 determined using the formula: SWC (%) = (FW−DW)/DW×100. Then we got an equation of linear
135 regression between the SWC and VWC.

136 Plants were allowed to acclimate to growth chamber conditions for one week before drought
137 treatments were imposed. Each line were randomly assigned to either the control group (n=4),
138 which was kept well-watered (100% ET), or to the drought treatment group (n=4), in which the
139 soil moisture was allowed to progressively decline from 0 d to 24 d. Each pots of drought treatment
140 were weighted every two days and VWC were also collected, then the water needed to add of each

141 pot was calculated to compensate for 30%-50% ET during the experiment over the 24 d period
142 (Fig. 1).
143
144 **Physiological measurements**
145 Leaf samples were collected for electrolyte leakage (EL) and relative water content (RWC)
146 measurements at day 0, 6, 12, 18 and 24 of drought stress. Leaf tissues were also sampled, frozen
147 with liquid N, and used for antioxidant emzyme and dehydrin analysis.
148 Leaf electrolyte leakage ( EL) was measured according to the method of Marcum [28] with
149 some modifications. The top 2$^{nd}$ or 3$^{rd}$ mature leaf blades were excised and cut into 2 cm segments.
150 After being rinsed 3 times with deionized $H_2O$, 0.2 g leaf segments were placed in a test tube
151 containing 20 mL deionized $H_2O$. The test tubes were agitated on a shaker for about 24 h and the
152 solution conductivity ($C_1$) was measured with a conductivity meter (SR60IC, VWR, Radnor, PA).
153 Leaf samples were then autoclaved at 120 $^oC$ for 30 min, and the conductivity of the solution
154 containing killed tissue was measured once the tubes were cooled down to room temperature ($C_2$).
155 The relative EL was calculated using the formula: EL (%) = ($C_1$/ $C_2$) ×100.
156 Leaf relative water content (RWC) was determined according to the method of Barrs and
157 Weatherley [29]. The Leaf RWC was calculated based on the following formula: RWC= (FW-
158 DW)/ (TW-DW) ×100, where FW is leaf fresh weight, DW is the dry weight of leaves after
159 drying at 85 $^oC$ for 3 d, and TW is the turgid weight of leaves after soaking in distilled water for
160 24 h at 20 $^oC$.
161
162 **Antioxidant enzyme activity**
163 Frozen leaf samples were ground in liquid nitrogen and homogenized in 50 mM sodium

164 phosphate buffer (pH 7.0) containing 2 mM EDTA, 5 mM β-mercaptoethanol and 4% (w/v)
165 polyvinylpyrrolidine-40 (PVP-40). The homogenate was centrifuged at 12,000×g for 30 min at
166 4 °C. The supernatant was used for assay of the antioxidant enzymes CAT, APX, and SOD.

167 Total SOD activity was determined by measuring its ability to inhibit the photochemical
168 reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries [30]
169 with minor modifications. The reaction solution (1 mL) contained 50 mM phosphate buffer (pH
170 7.8), 0.1 mM EDTA, 13 mM methionine, 65 μM NBT and 1.3 μM riboflavin, and 30 μL SOD
171 extract. A solution containing no enzyme solution was used as the control. Test tubes were
172 irradiated under fluorescent lights 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 25 ºC for 10 min. The absorbance of each
173 solution was measured at nm using a spectrophotometer, and one unit of enzyme activity was
174 defined as the amount of enzyme that would inhibit 50% of NBT photoreduction.

175 The CAT activity was determined using the method of Chance and Maehly[31] with
176 modifications. For CAT, the reaction solution (1 mL) contained 50 mM phosphate buffer (pH 7.0),
177 15 mM $H_2O_2$, and 30 μL of extract. The reaction was initiated by adding the enzyme extract.
178 Changes in absorbance at 240 nm were read in 1 min using a spectrophotometer ($\epsilon$ = 39.4 $M^{-1}$
179 $cm^{-1}$).

180 The APX activity was assayed by recording the decrease in absorbance at 290 nm for 1 min.
181 The 1.5-mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM
182 ascorbic acid, 0.1 mM EDTA, 0.1 mM $H_2O_2$, and 0.15 mL of enzyme. The reaction was started
183 with the addition of 0.1 mM $H_2O_2$ [32].

184

185 **Lipid peroxidation**

186 Lipid peroxidation was measured in term of leaf MDA content [33]. A 1 ml aliquot of

187 supernatant was mixed with 4 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid.
188 The mixture was heated at 100 °C for 30 min, quickly cooled, and then centrifuged at 10000 *g* for
189 10 min. The absorbance of the supernatant was read at 532 nm. The unspecific turbidity was
190 corrected by A600 subtracting from A530. The concentration of MDA was calculated using an
191 extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.
192
193 **Antioxidant isozymes**
194 The procedure of protein extraction was the same as for antioxidant enzymes. The extracts (15
195 μL) for SOD, CAT and APX were loaded on each gel. Native polyacrylamide gel electrophoresis
196 (PAGE) was performed using a Mini-Protean system (Bio-Rad Laboratories, Hercules, CA) at 4
197 $^{O}$C, 120 V for 90 min, except that SDS was omitted. For SOD and APX, enzyme extracts were
198 subjected to native PAGE with 10% resolving gel and 3% stacking gel and CAT was detected on
199 7% resolving gel and 4% stacking gel.
200 The total activity of SOD was revealed using the method of Beauchamp and Fridovich[34] with
201 some modifications. The gels were incubated in 50 mM potassium phosphate buffer (pH 7.5)
202 containing 2.5 mM NBT in dark for 25 min. After being washed twice with the same buffer, the
203 gels were soaked in 50 mM potassium phosphate buffer (pH 7.5) containing 30 μM riboflavin and
204 0.4% N,N,N,N-tetramethylethylenediamine 235 (TEMED) in the dark for 40 min. The gels were
205 then illuminated for 10 min with gentle agitation until appearance of enzyme bands and were
206 transferred to 1% (v/v) acetic acid to stop the reaction.
207 The CAT isozyme staining was performed according to He and Huang[35] and Woodbury *et al*.
208 [36] with modifications. After finishing each run, the gel was washed in three changes of distilled
209 water for a total of about 6 min to remove the buffer from the gel surface where staining occurred.

210 The gels were incubated in 5 mM $H_2O_2$ for 10 min in the dark, and then in the stain mixture
211 containing 1% (w/v) potassium ferricyanide $K_3[Fe(CN_6)]$ and 1% (w/v) ferric chloride ($FeCl_3$),
212 (equal volume of 2% (w/v), added sequentially) until the light yellow bands (CAT isoenzymes)
213 were visible.

214 The activity of APX was detected using the method of Lopez-Huertas *et al*. [37] with some
215 modifications. The gels were pre-incubated in 50 mM sodium phosphate buffer with 4 mM
216 ascorbate and 2 mM $H_2O_2$ for 20 min. After briefly being washed with 50 mM potassium
217 phosphate buffer (pH 7.0), the gels were stained in 50 mM potassium phosphate buffer (pH 7.8)
218 containing 28 mM TEMED and 1.25 mM NBT until the bands were clearly visible. The gels were
219 then washed with distilled water to stop the reaction.

220

221 **SDS-PAGE and western blot**

222 Frozen leaf samples were ground in liquid nitrogen and re-suspended in100 μl 3 × Laemmli
223 buffer containing 16% β-mercaptoethanol.The tissue was then boiled for 10 min and pelleted at a
224 high speed for 10 min.Twenty micro liters of protein extract was applied to and separated on a 10%
225 SDS-PAGE gel. The proteins were blotted to a PVDF membrane using a Bio-Rad Trans-Blot R
226 TurboTM Transfer System. The membrane was blocked with 5% nonfat skim milk in 1 × Tris-
227 saline buffer supplemented with 0.5% Tween20 (1 × TBST). After a brief rinse with TBS, the
228 membrane was incubated in TBS with a dehydrin polyclonal antibody raised from rabbit (Assay
229 Designs) at a dilution of 1:250 for 1.5 h. Next, the membrane was rinsed in TBS containing 0.5%
230 Tween 20 (TBS-T) four times and then placed for 1 h in a solution of goat antirabbit IgG (dilution
231 1:17500) conjugated to alkaline phosphatase (Sigma). The membrane was rinsed in TBS-T four
232 times.The chemiluminescent signals were exposed to autoradiography film(Genesee Scientific,

233 SanDiego,CA) using a Kodak film processor (Kodak,A Walsh Imaging, Inc,Pompton Lakes, NJ).

234

235 **RNA extraction and quantitative reverse transcription PCR (qRT-PCR)**

236 Total RNA was extracted from 150 mg of leaf tissue using RNeasy plant mini RNA kit (50)
237 (Qiagen, Valencia, CA), and RNA samples were further treated with DNase (Promega, Madison,
238 WI, USA) to eliminate DNA contamination. Integrity of RNA was confirmed with miniaturized
239 gel electrophoresis with the Agilent Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA).
240 cDNA was synthesized using the DyNAmo cDNA Synthesis Kit (New England Biolabs,
241 Ipswich,MA, USA). The qRT-PCR analysis primer pairs of the corresponding genes were
242 designed according to sequences obtained from the Phytozyme website (table 1). Each 20-µl
243 reaction, which contained 15 ng of random hexamers/µl, 10 IU of Moloney murine leukemia virus
244 RNase $H^+$ reverse transcriptase solution (Thermo Fisher Scientific), and appropriate buffer
245 containing deoxynucleoside triphosphates (dNTPs) and $MgCl_2$ in a final concentration of 5 mM
246 (1×; Thermo Fisher Scientific), was incubated at 25°C for 10 min and at 37°C for 30 min,
247 inactivated at 85°C for 5 min, and finally chilled to 4°C. Two replicate RT reactions were made
248 for each RNA sample.

249

250 **Experimental design and statistical analysis**

251 The experiment was a 4×2 factorial combination (two switchgrass cultivars, and two drought
252 levels: well-watered and drought treatment) in a complete block design (one treatment of one
253 species served as the block) with four replications. All data were subjected to analysis of
254 variance (ANOVA, SAS 8.1, SAS Institute Inc., Cary, NC). The treatment means were separated
255 using Fisher's protected least significant difference (LSD) test at 5% probability level.

256

257 **Results**

258 **Effects of drought stress on physiological parameters**

259 Drought stress reduced leaf RWC, increased leaf EL and MDA regardless of cultivars (Fig. 2,
260 3 & 4). There were significant differences in leaf RWC, leaf EL and MDA in the two cultivars
261 under drought stress conditions when compared to the controls at 18 d and 24 d. Leaf RWC, leaf
262 EL and MDA of Dacotah decreased or increased sharply at both 18 d and 24 d of drought
263 treatments. Alamo had a relatively higher leaf RWC and lower leaf EL and MDA than Dacotah at
264 12 d, 18 d and 24 d of drought stress.

265 **Effects of drought stress on antioxidant enzymes activity**

266 The SOD, CAT and APX activities were progressively enhanced in the two cultivars with
267 duration of drought stress when compared to their control (Figs. 5, 6, and 7), Alamo had greater
268 SOD, APX and CAT activities than Dacotah at 24 d of drought treatment.

269 **Effects of drought stress on antioxidant isozyme**

270 Our activity staining visualized four SOD isozymes (SOD1-SOD4) in two cultivars (Fig. 8).
271 Alamo and Dacotah increased SOD1 abundance under drought stress compared to control, both
272 cultivars increased SOD2-SOD3 abundance under drought stress compared to control, however,
273 only Dacotah decreased SOD4 abundance under drought stress compared to control. Alamo had
274 relative higher SOD3- SOD4 abundance in response to drought stress compared to Dacotah. Only
275 one isoform of APX was identified in two cultivars (Fig. 9), both cultivars increased APX1
276 abundance under drought stress compared to control, Alamo had relative higher APX1 abundance
277 in response to drought stress when compared to Dacotah.

278 **Effects of drought stress on dehydrin**

279 Drought stress increased dehydrin accumulation in both cultivars, but Alamo had higher
280 abundance of 53 KDa and 18 KDa dehydrin than Dacotah (Fig. 10), however, no dehydrin
281 accumulation of 18 KDa was found in Dacotah.

282 **Antioxidant, dehydrin, heat shock protein (HSP) and aquaporin genes expression**

283 The level of *PvAPX2*, *PvCAT1*, *PvERD1* and *PvPIP1;5* mRNA significantly increased in
284 Alamo compared to Dacotah at 24 d of drought stress (Fig. 11), however, the level of *PvSOD1*
285 mRNA significantly increased in Dacotah at 24 d of drought stress, no significant difference in
286 level of *PvHSP90* mRNA between Alamo and Dacotah at 24 d of drought stress.

287

288 **Discussion**

289 Our previous study found that, Alamo ranked #4 in drought tolerance and Dacotah ranked #48
290 among 49 switchgrass lines, Alamo is a representative of good drought tolerant cultivars and
291 Dacotah is a representative of drought sensitive ones [26]. The results of this study agreed with
292 our previous study, showed that drought stress caused cellular and leaf damage to switchgrass as
293 indicated by decreased leaf RWC, increased EL and MDA. Alamo had higher RWC, *PvPIP1;5*
294 relative gene expression level, lower EL and MDA content than Dacotah at 24 d of drought
295 treatment, indicating that drought stress resulted in more severe damage to cell membranes in
296 Docatah relative to Alamo at 24 d of drought treatment.

297 When plants are subjected to water deficit, drought tolerant cultivars have better Tr and $g_s$ and
298 gas exchange and less oxidative stress, changes in antioxidants genes expression and activities
299 may improve antioxidant defense system and ROS scavenging [38]. SOD is regarded as the key
300 enzyme in the ROS scavenger system because it catalyzes superoxide free radical dismutation into
301 $H_2O_2$ and $O_2$, which is the first step of scavenging ROS [39]. APX and POD scavenge $H_2O_2$ and

302 reduce ROS toxicity. In this study, we choose relative drought-tolerant cultivar Alamo, and relative
303 drought-sensitive cultivar Dacotah to study the drought tolerance mechanisms of drought
304 associated with antioxidant defense. Our results showed that, drought stress increase SOD, CAT
305 and APX in two cultivars especially at 18 d and 24 d treatment. Alamo had significant higher SOD,
306 CAT, APX activities and *PvAPX2*, *PvCAT1* relative gene expression level than Dacotah. R
307 Khanna-Chopra *et al*. [40] found that drought-resistant wheat maintained favorable water relations
308 and lower $H_2O_2$ accumulation during severe water stress conditions due to systematic increase of
309 antioxidants such as SOD, APX, CAT [41]. Pallavi Sharma *et al*. [11] reported that drought stress
310 caused oxidative damage in rice species as exhibited by an increase in $O^{\bullet-}_2$ and $H_2O_2$, but drought
311 tolerant rice maintained higher antioxidant enzyme activities (SOD, APX) compared with drought-
312 sensitive rice. These results indicated that antioxidant defense plays an important role in
313 improvement of drought tolerance in plants, drought tolerant cultivars may have greater ROS-
314 scavenging capacity to suppress ROS-induced injury during abiotic stress.

315 Antioxidant isozymes are known to play a vital role in plant drought tolerance. In this study,
316 antioxidant isozyme diversity of two switchgrass cultivars were investigated to identify band
317 profiles as biochemical marker for the drought tolerance. Drought stress increased abundance of
318 SOD1-SOD3 and APX1 isozyme in two cultivars at 24 d of drought stress, however, Dacotah had
319 relatively lower SOD4 and APX1 abundance when compared to Alamo at 24 d of drought stress.
320 The results of this study indicate that the drought tolerant cultivar Alamo had greater ability to
321 scavenge ROS than the drought sensitive Dacotah. Our results agreed with the previous studies,
322 which showed that various drought-tolerant plants present different isozyme patterns during
323 drought stress conditions [42, 43].

324 Drought induced-accumulation of dehydrin proteins has been associated with drought tolerance

325 in many plant species [44-46]. In this study, two dehydrin polypeptides (53 KDa and 18 KDa)
326 were detected in Alamo under drought stress conditions at 24 d of drought treatment. Interestingly,
327 only one dehydrin polypeptides (53 KDa) was detected in Dacotah, that could be caused by the
328 different drought tolerant ability between the two cultivar under drought stress. Consistently,
329 Alamo had significant higher *PvERD1* relative gene expression level than Dacotah.

330 In summary, drought stress caused damage to switchgrass as evidenced by decreased RWC,
331 increased leaf EL and MDA content due to drought stress treatment. Alamo had relatively higher
332 RWC, lower EL and less MDA content when compared to Dacotah at 24 d of drought stress. SOD,
333 APX and CAT activities increased during drought stress at 18 d and 24 d. Alamo had higher SOD,
334 APX and CAT activities, greater abundance of SOD1-SOD3 and APX1 isozyme than Dacotah
335 under drought stress. Alamo had greater abundance of two dehydrin polypeptides (53 KDa and 18
336 KDa) under drought stress conditions at 24 d than Dacotah. qRT-PCR showed that antioxidant
337 gene *PvAPX2*, *PvCAT1*, dehydrin gene *PvERD1*, and aquaporin gene *PvPIP1;5* instead of heat
338 shock protein gene *PvHSP90* and *PvSOD1*, are the key genes contribute to the drought tolerance
339 of Alamo, although Alamo had higher SOD activity and SOD isozyme abundance. Our results
340 suggest antioxidant and dehydrin expression are associated with drought tolerance in switchgrass.
341 Our results also suggest that selection and use of cultivars with greater antioxidant enzyme
342 activities and more abundant isozymes under drought stress may be a practical approach to
343 improve switchgrass drought tolerance.

344

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455

456

457 Table 1 Real-time polymerase chain reaction primers used for analysis of antioxidant, dehydrin

458 and aquaporin gene expression in two switchgrass cultivars contrasting in drought tolerance

| No. | Gene name | Transcript_identifier | Primer_fw_sequence | Primer_fw_Tm | Primer_rev_sequence | Primer_rev_Tm | Amplicon_length |
|---|---|---|---|---|---|---|---|
| 1 | *PvAPX2* | Pvr.568 | TTCCTGATGCCACCCAAGGTTC | 63.169 | AATGTCCTGGTCGCTCAAACCC | 63.455 | 77 |
| 2 | *PvCAT1* | Pvr.13277 | AGGCAAGAGCGGTTCATCAAGAG | 63.398 | ATGCTCTGATCTCGTGGGTGAG | 62.211 | 73 |
| 3 | *PvERD1* | Pvr.94 | AGCTTTGCTGATCTGTTCTGCTG | 62.025 | CATGATCGCCGGAACTAAAGGC | 62.292 | 60 |
| 4 | *PvHSP90* | Pvr.11953 | TCGATTGAGCGAGCTGAGTGTG | 63.239 | ATCGAAGGCAACCTCGAACTGG | 63.227 | 68 |
| 5 | *PvPIP1;5* | Pvr.149 | AATTGGGTTCGCCGTGTTCCTG | 64.255 | TGTAGATGATGGCAGCGCCAAG | 63.613 | 98 |
| 6 | *PvSOD1* | Pvr.1363 | ATTGGCCGAGCTGTAGTTGTCC | 63.245 | TAAGCTCATGTCCACCCTTGCC | 62.958 | 64 |

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475 **Figure legends**

476 Fig.1. Effects of drought stress on soil water content (SWC). Vertical bars indicate LSD value
477 (P=0.05).

478 Fig.2. Effects of drought stress on relative water content (RWC) of two switchgrass cultivars
479 TEM-SEC, Alamo, Caddo and Dacotah. Vertical bars indicate LSD value (P=0.05).

480 Fig. 3. Effects of drought stress on leaf electrolyte leakage (EL) of two switchgrass cultivars EM-
481 SEC, Alamo, Caddo and Dacotah. Vertical bars indicate LSD value (P=0.05).

482 Fig. 4. Effects of drought stress on malondialdehyde (MDA) of two switchgrass cultivars EM-SEC,
483 Alamo, Caddo and Dacotah. Vertical bars indicate LSD value (P=0.05).

484 Fig. 5. Effects of drought stress on superoxide dismutase (SOD) of two switchgrass cultivars EM-
485 SEC, Alamo, Caddo and Dacotah. Vertical bars indicate LSD value (P=0.05).

486 Fig. 6. Effects of drought stress on ascorbate peroxidase (APX) of two switchgrass cultivars EM-
487 SEC, Alamo, Caddo and Dacotah. Vertical bars indicate LSD value (P=0.05).

488 Fig. 7. Effects of drought stress on ascorbate catalase (CAT) of two switchgrass cultivars EM-
489 SEC, Alamo, Caddo and Dacotah. Vertical bars indicate LSD value (P=0.05).

490 Fig. 8. Changes in superoxide dismutase (SOD) isoforms of two switchgrass cultivars EM-SEC,
491 Alamo, Caddo and Dacotah under control (well-watered) and drought stress conditions (24 d).
492 Equal amounts (15 μL) were loaded in each lane.

493 Fig. 9. Changes in ascorbate peroxidase (APX) isoforms of two switchgrass cultivars EM-SEC,
494 Alamo, Caddo and Dacotah under control (well-watered) and drought stress conditions (24 d).
495 Equal amounts (15 μL) were loaded in each lane.

496 Fig. 10. Immunoblots of dehydrin expression in two switchgrass cultivars EM-SEC, Alamo,
497 Caddo and Dacotah under drought stress (24 d).

498 Fig. 11. Relative transcript levels of *PvAPX2*, *PvCAT1*, *PvERD1*, *PvHSP90*, *PvSOD1* and
499 PvPIP1;5 genes of two switchgrass cultivars (drought-tolerant Alamo and drought-sensitive
500 Dacotah) under well-watered and drought stress (24 d). Each bar represents the mean of three
501 independent replicates with standard error. Different letters of a-c indicates the statistic
502 difference at 0.05 level.

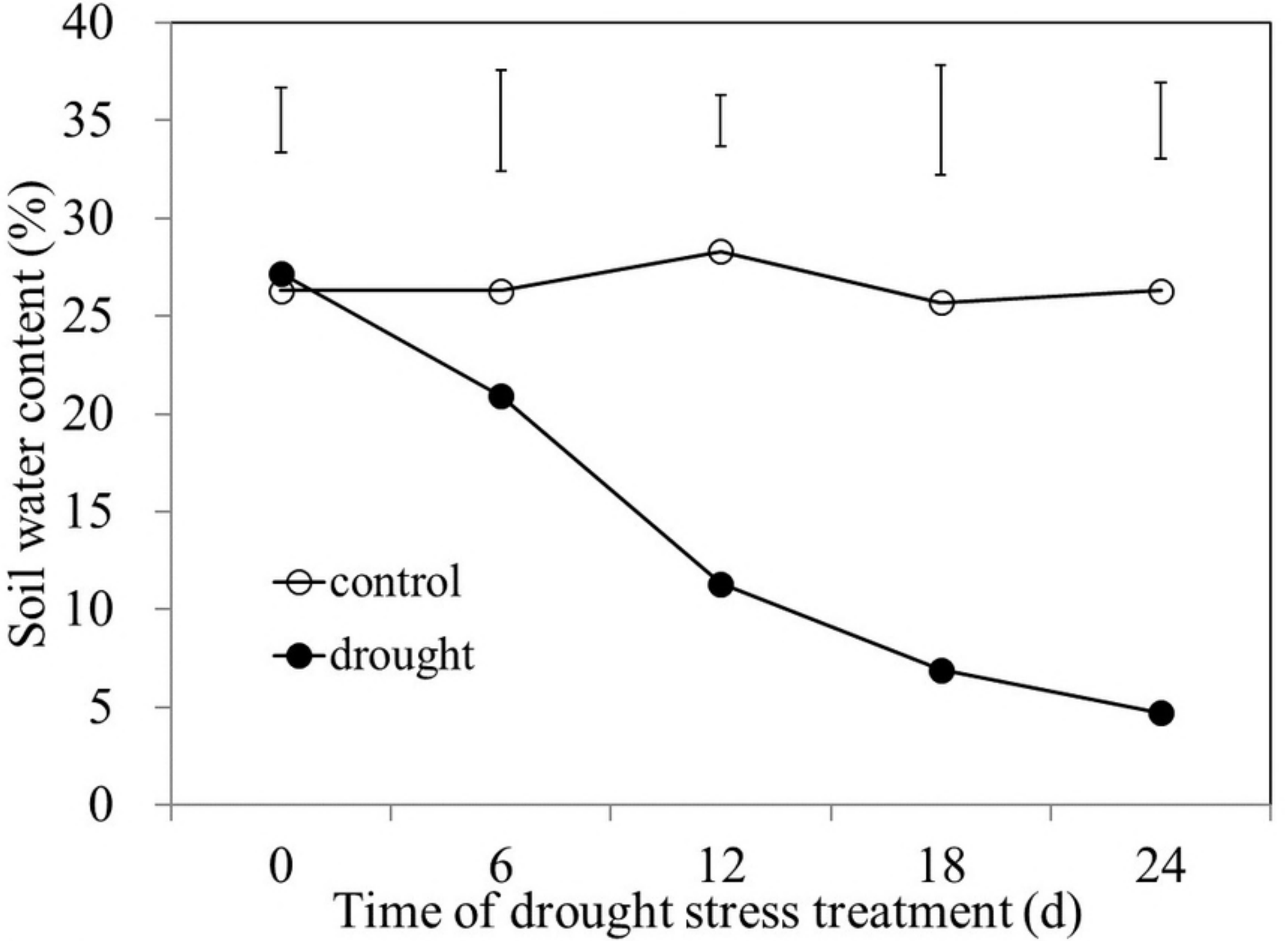
40
35
30
25
20
15
10
5
0
Soil water content (%)
control
drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.1

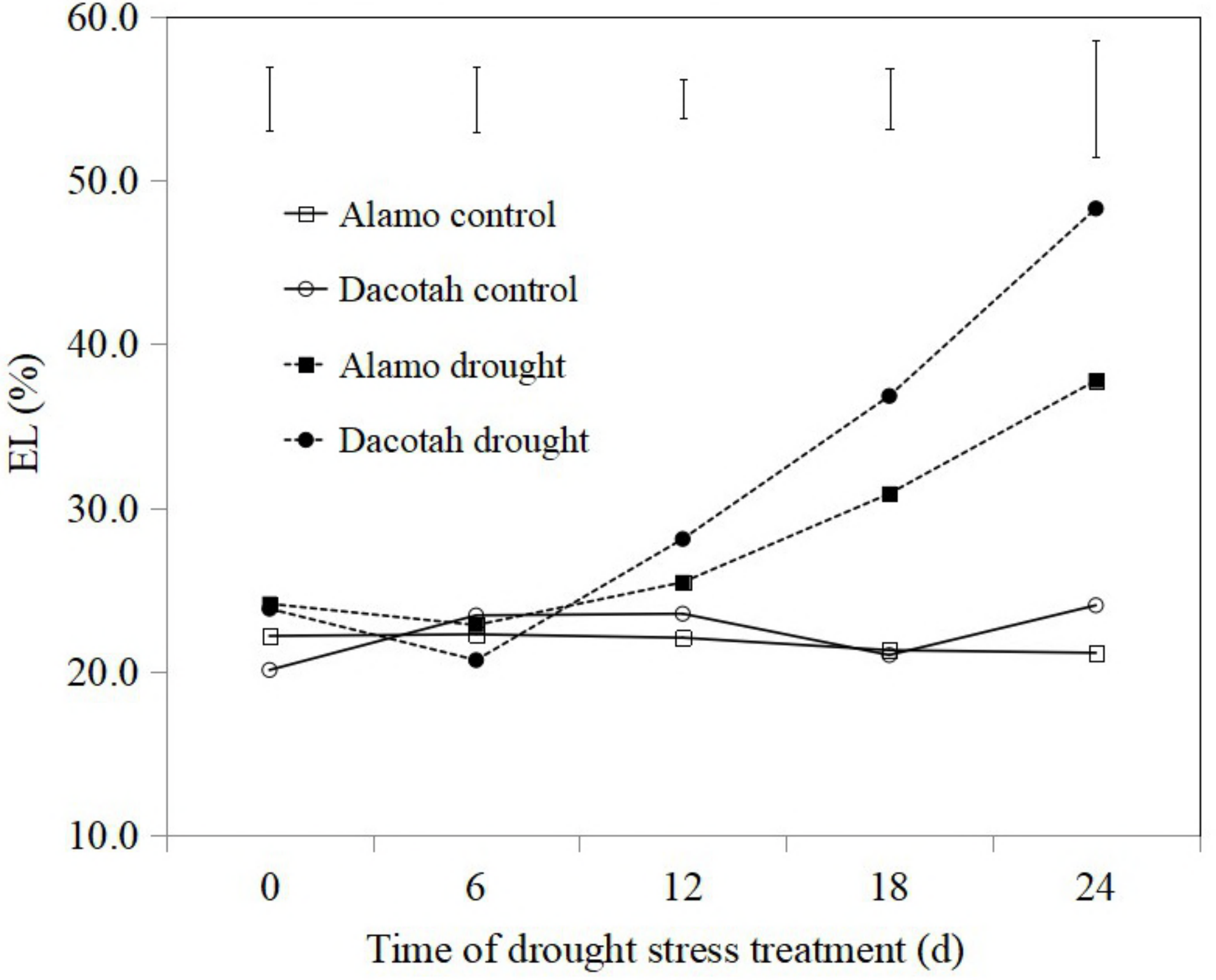
60.0
50.0
40.0
30.0
20.0
10.0
EL (%)
Alamo control
Dacotah control
Alamo drought
Dacotah drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.2

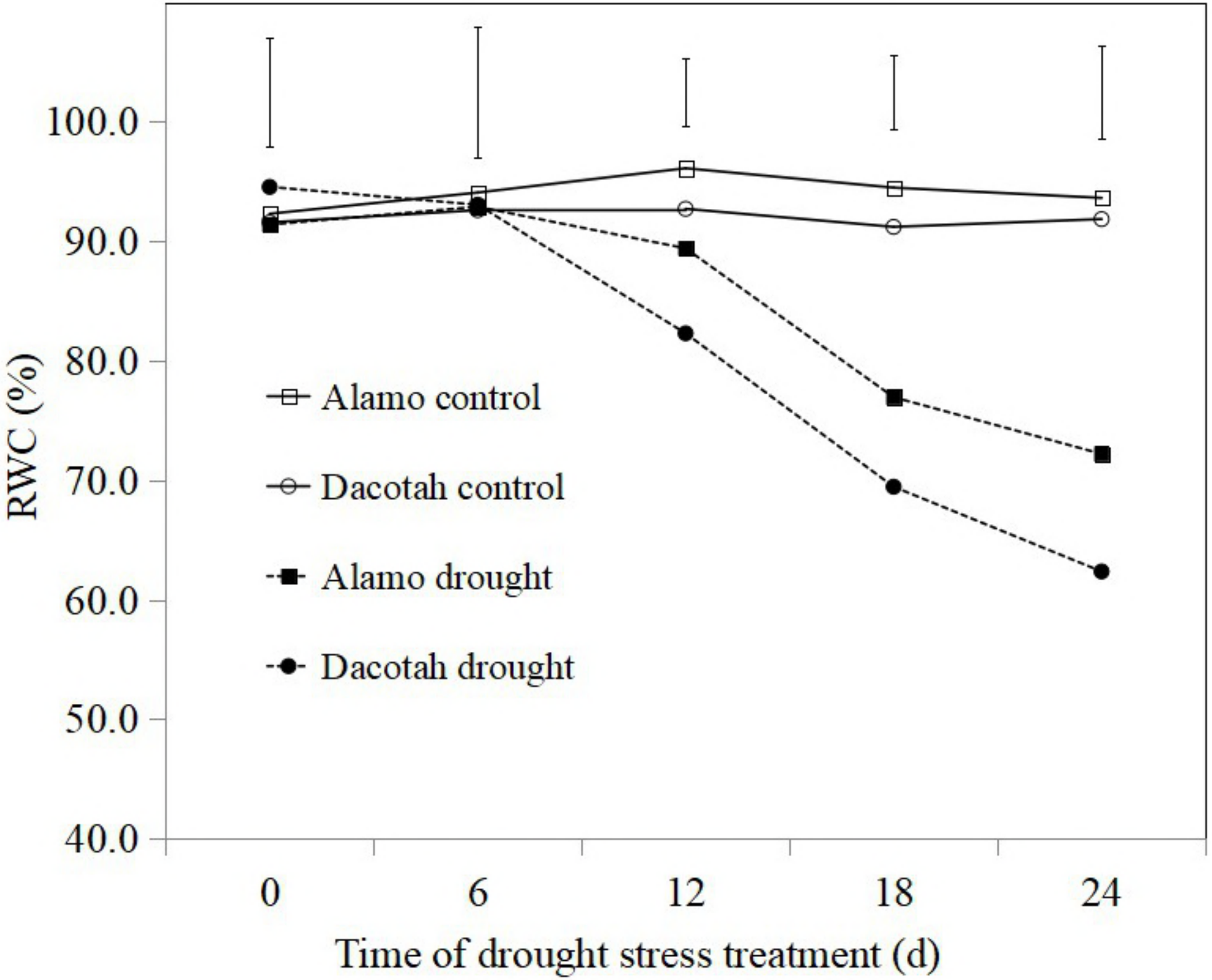
100.0
90.0
80.0
70.0
60.0
50.0
40.0
RWC (%)
Alamo control
Dacotah control
Alamo drought
Dacotah drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.3

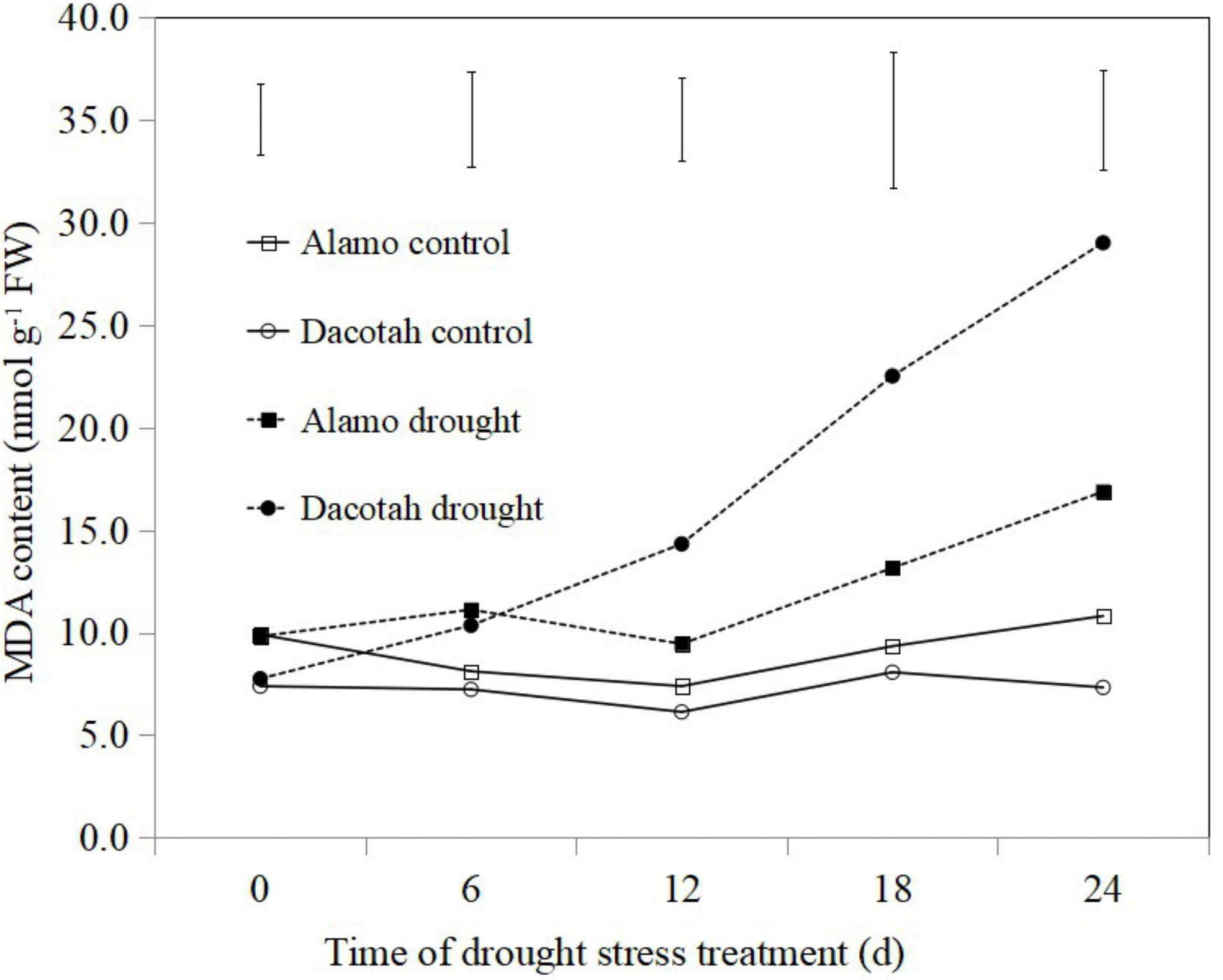
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
MDA content (nmol g-1 FW)
Alamo control
Dacotah control
Alamo drought
Dacotah drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.4

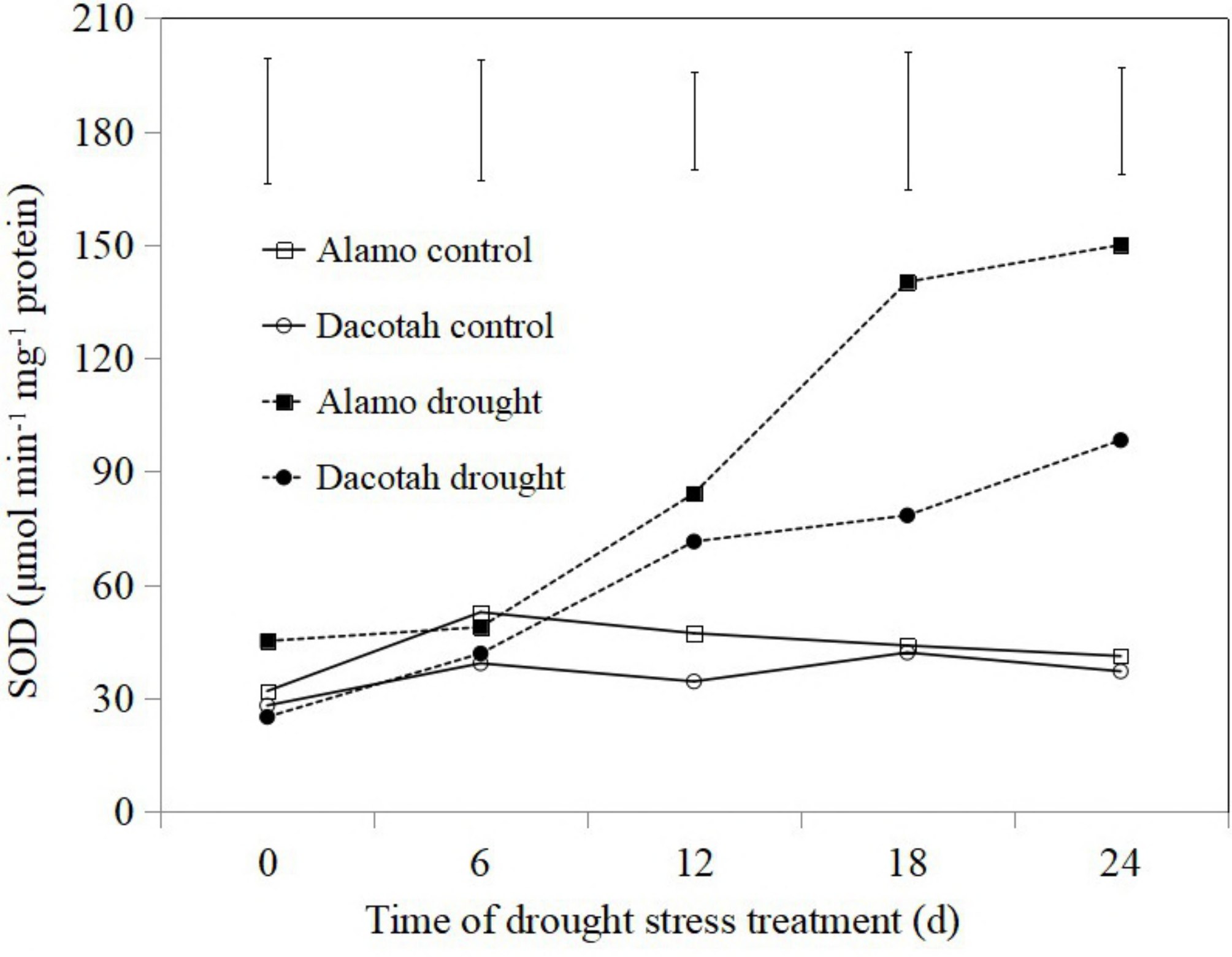
210
180
150
120
90
60
30
0
SOD (μmol min-1 mg-1 protein)
Alamo control
Dacotah control
Alamo drought
Dacotah drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.5

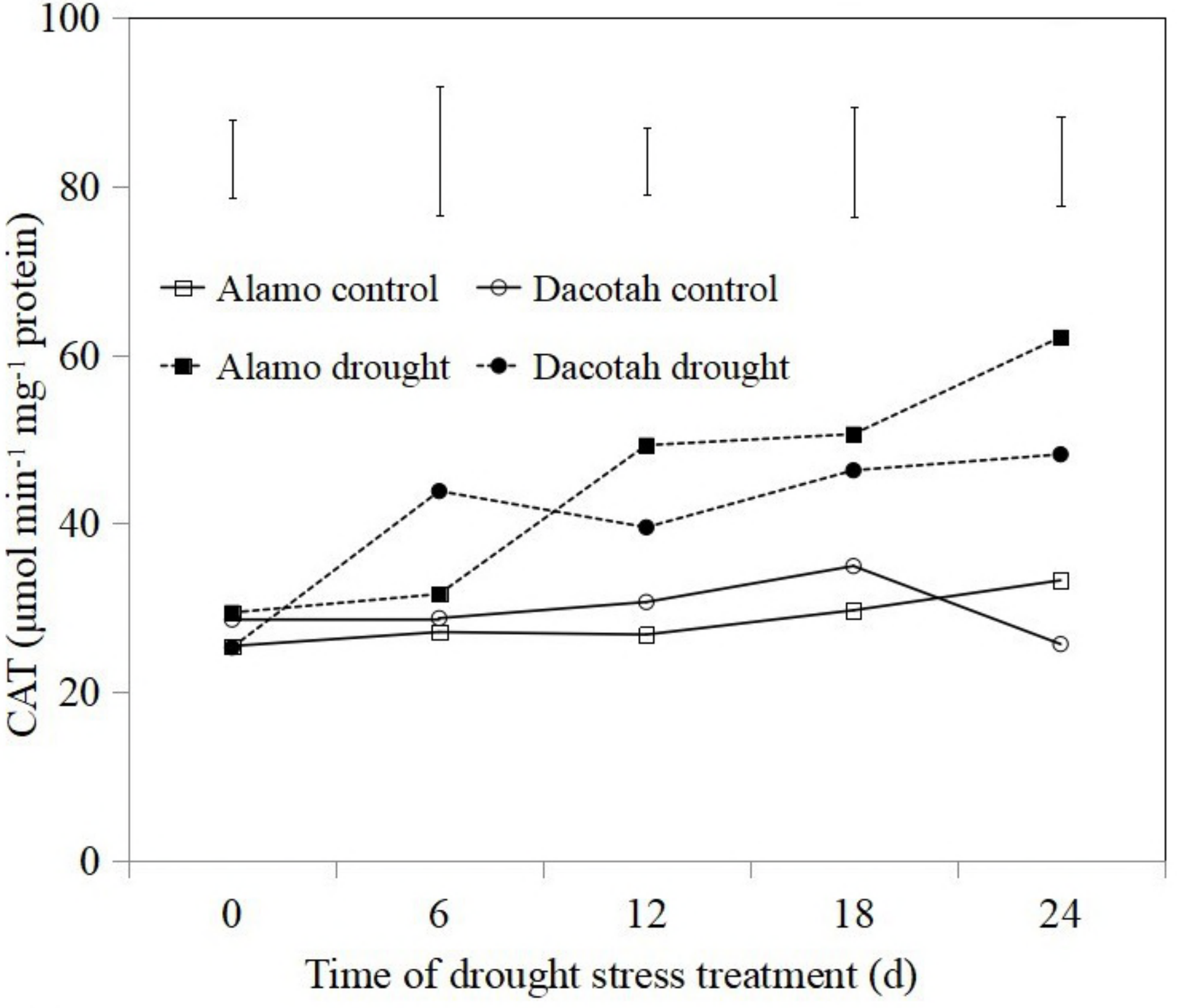
100
80
60
40
20
0
CAT (μmol min-1 mg-1 protein)
Alamo control
Dacotah control
Alamo drought
Dacotah drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.6

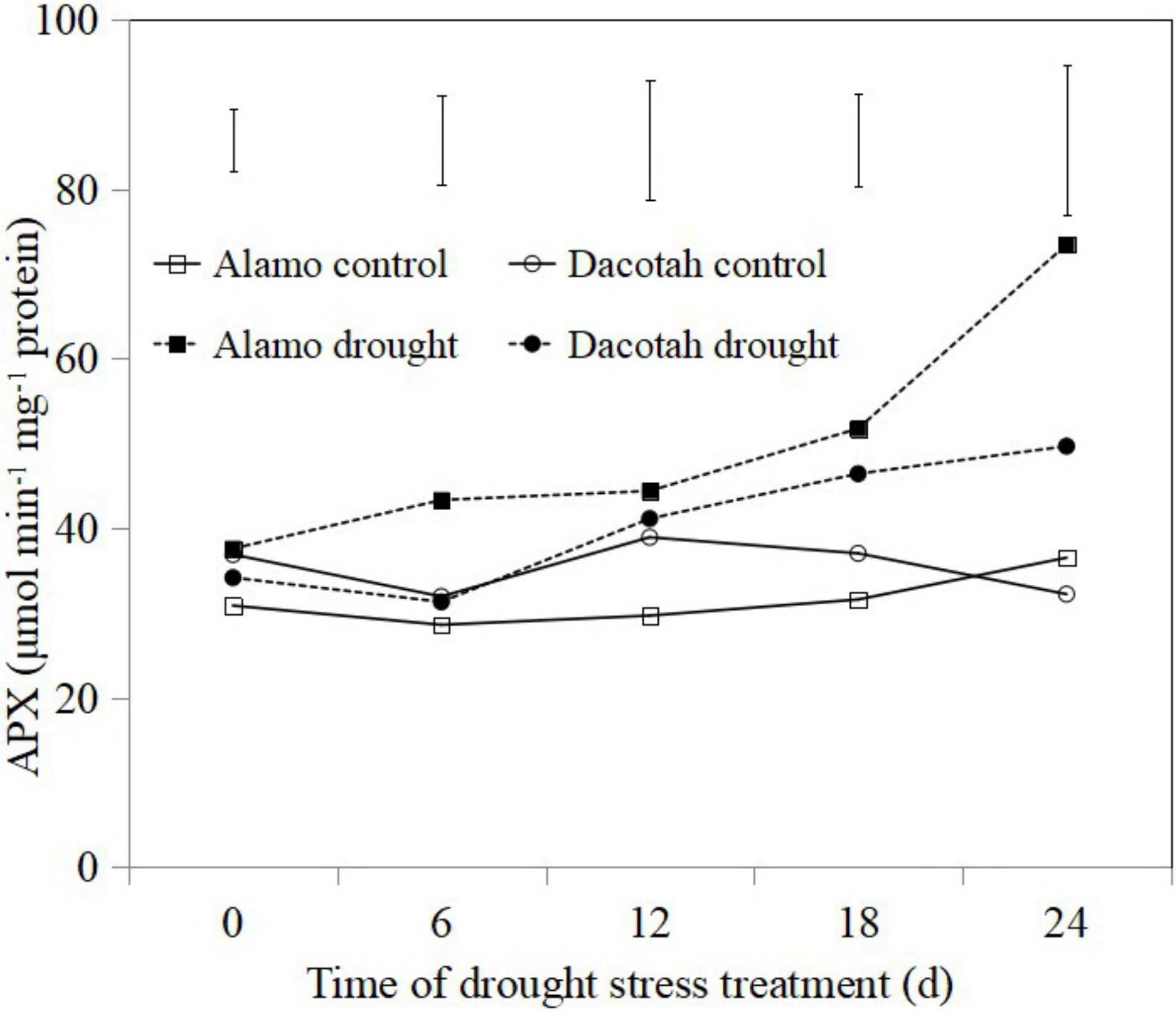
100
80
60
40
20
0
APX (µmol min-1 mg-1 protein)
Alamo control
Dacotah control
Alamo drought
Dacotah drought
0
6
12
18
24
Time of drought stress treatment (d)

Fig.7

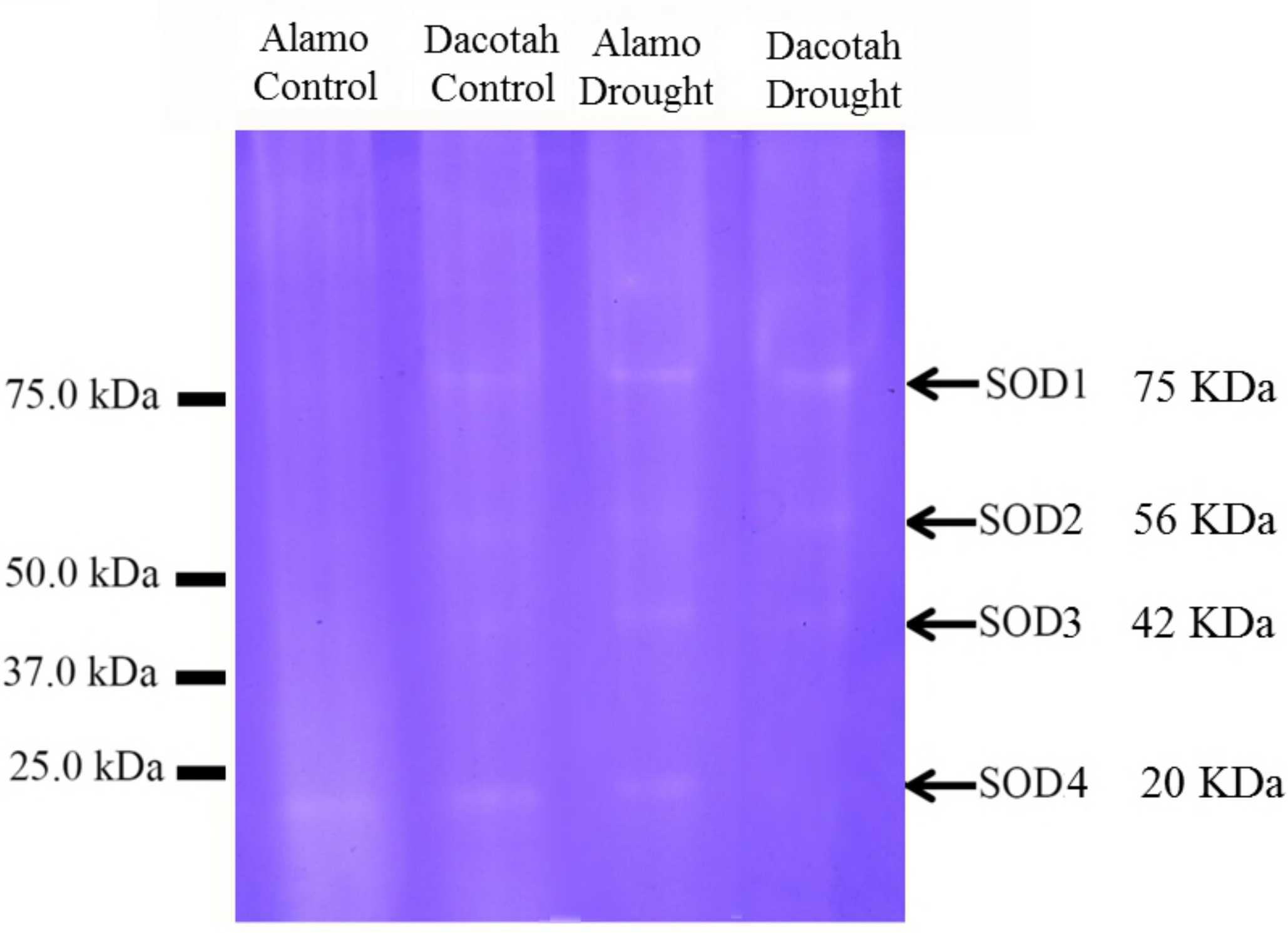
Alamo Control
Dacotah Control
Alamo Drought
Dacotah Drought
75.0 kDa
50.0 kDa
37.0 kDa
25.0 kDa
SOD1 75 KDa
SOD2 56 KDa
SOD3 42 KDa
SOD4 20 KDa

Fig.8

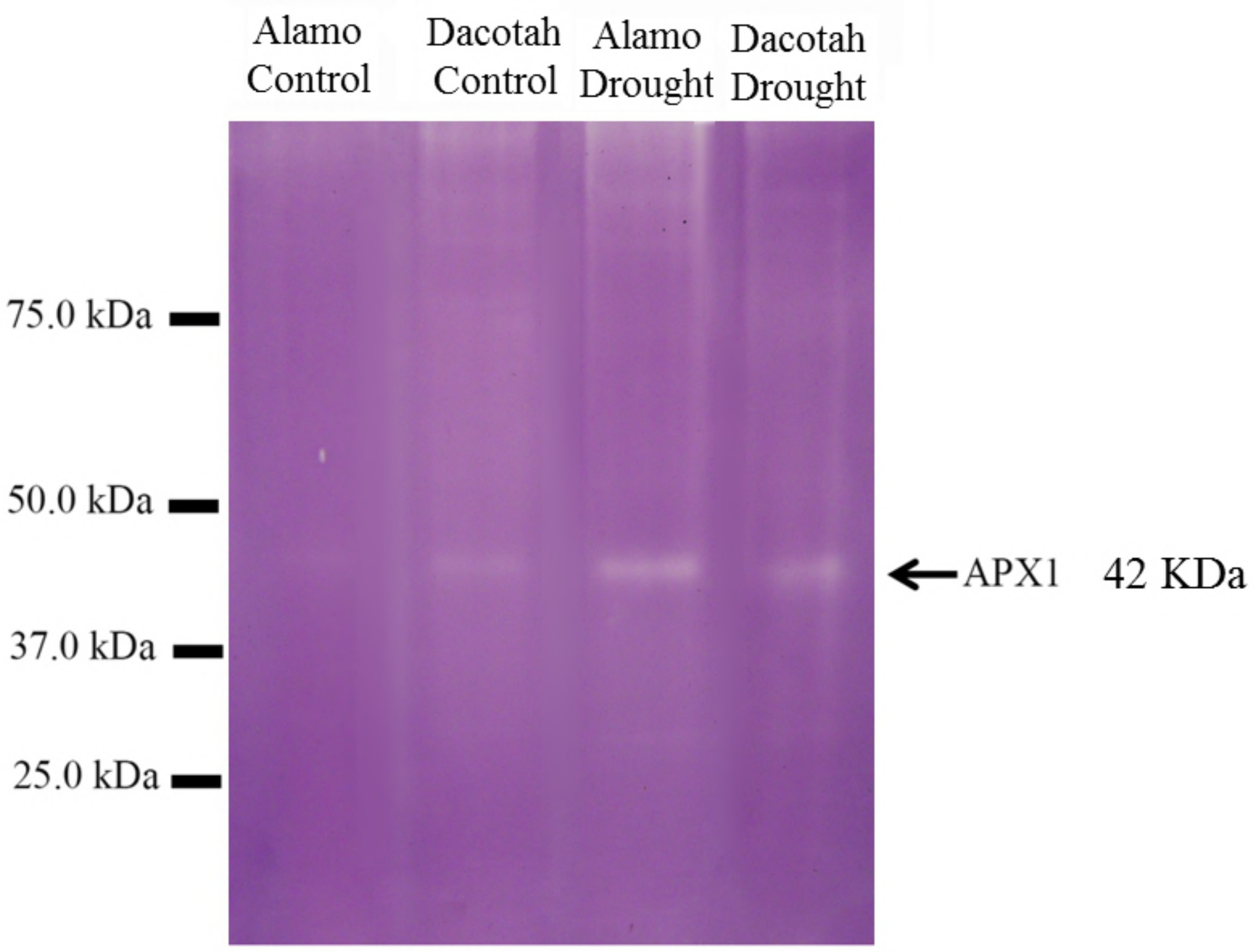
Alamo Control
Dacotah Control
Alamo Drought
Dacotah Drought
75.0 kDa
50.0 kDa
37.0 kDa
25.0 kDa
APX1 42 KDa

Fig.9

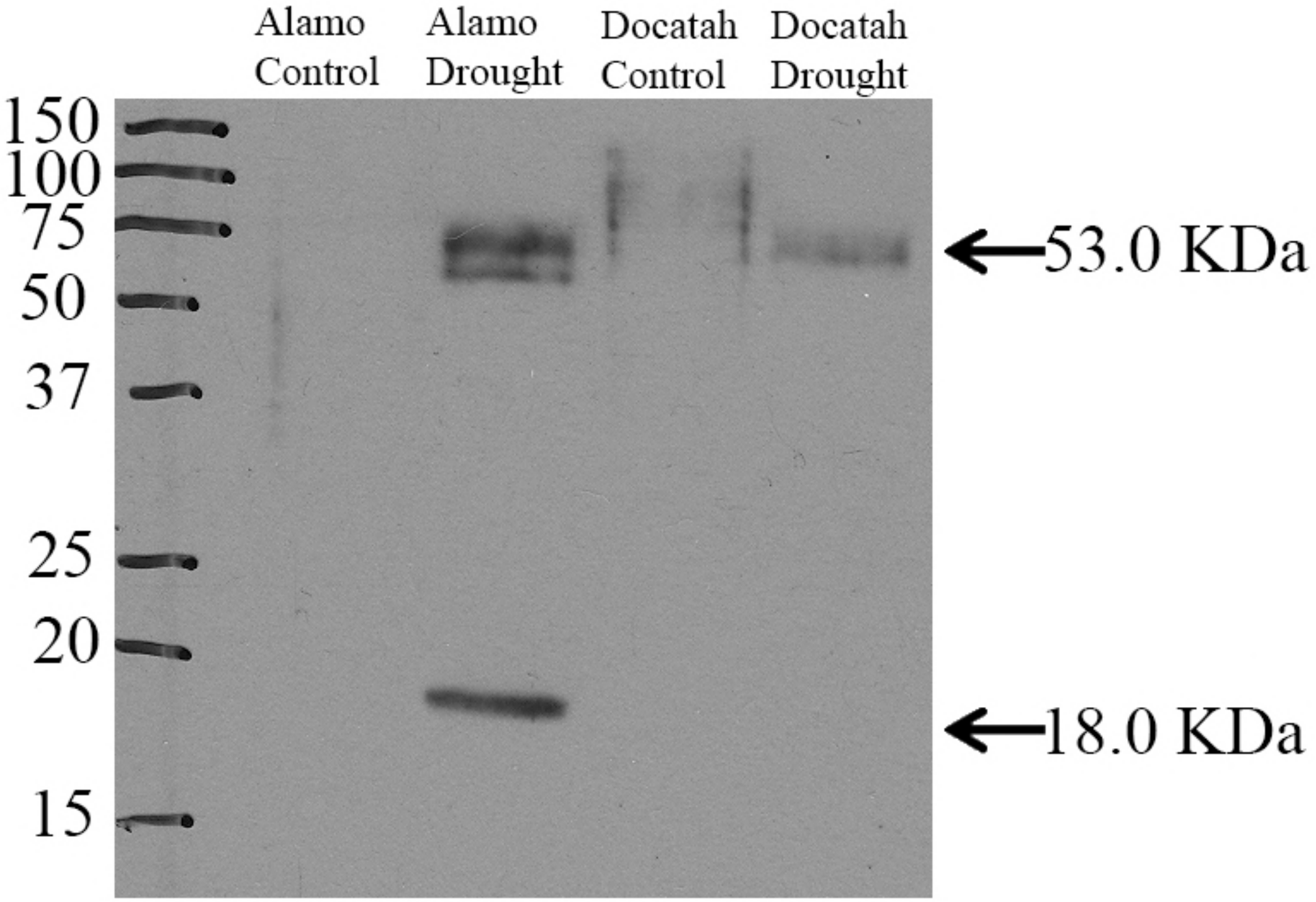
Alamo Control
Alamo Drought
Docatah Control
Docatah Drought
150
100
75
50
37
25
20
15
53.0 KDa
18.0 KDa

Fig.10

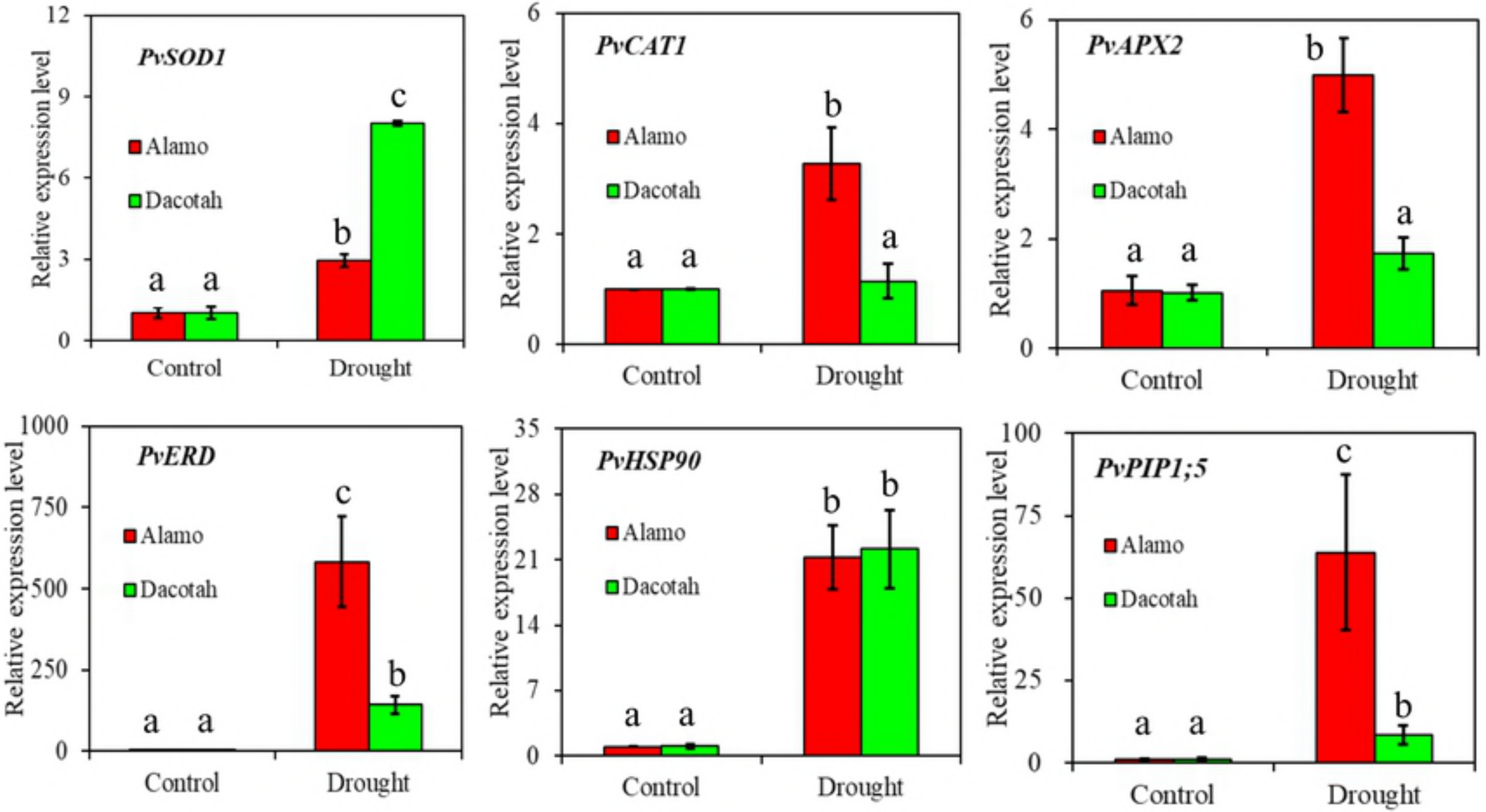
PvSOD1
Alamo
Dacotah
Relative expression level
12
9
6
3
0
a a
b
c
Control
Drought
PvCAT1
Alamo
Dacotah
Relative expression level
6
4
2
0
a a
b
a
Control
Drought
PvAPX2
Alamo
Dacotah
Relative expression level
6
4
2
0
a a
b
a
Control
Drought
PvERD
Alamo
Dacotah
Relative expression level
1000
750
500
250
0
a a
c
b
Control
Drought
PvHSP90
Alamo
Dacotah
Relative expression level
35
28
21
14
7
0
a a
b
b
Control
Drought
PvPIP1;5
Alamo
Dacotah
Relative expression level
100
75
50
25
0
a a
c
b
Control
Drought

Fig.11